19. (Amended) A method of regulating the expression of a recombinant nucleic acid sequence encoding a polypeptide which is immunogenic in a mammal and to which the mammal has already made an immune response, the method comprising introducing a cell into said mammal, said cell transformed in vitro with a vector comprising a nucleic acid encoding said polypeptide, said nucleic acid operably linked to a tetracycline-regulatable promoter; wherein prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro, and altering the concentration of tetracycline or an analog thereof to which the cell is exposed in said mammal, so as to achieve in said mammal expression of said immunogenic polypeptide that is altered in the presence or absence of tetracycline or an analog thereof.

Amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages ii - v).

REMARKS

Claims 1-3, 5-6, 8-9, 13-14, 16 and 18-20 are currently pending in the application. Claims 1, 14 and 18-19 are amended. The amendments find support in the specification and are discussed in the relevant sections below. No new matter is added.

Priority Claim

Certified copies of United Kingdom applications 9718872.6, filed September 6, 1997, and 9723448.8, filed November 7, 1997, to which the instant application claims priority, are filed herewith.

Specification

The description of Fig. 2 has been amended to refer to Figures 2A and 2B, as requested by the Examiner.

Claim Objections

Claims 1-3, 5-6, 8-9, 13-14, 16 and 18-20 are objected to because of informalities in the claims.

Claims 1, 14 and 19 have been objected to because they should read "encoding an immunogenic polypeptide" to reflect the "said immunogenic polypeptide" in the body of the claim.

Applicants respectfully submit that antecedent basis exists for the term "immunogenic polypeptide". As stated in the Manual of Patent Examining Procedure (MPEP),

> the failure to provide explicit antecedent basis for terms does not always render a claim indefinite. If the scope of a claim would be reasonably ascertainable by those skilled in the art, then the claim is not indefinite. *Ex parte Porte*r, 25 USPQ2d 1144, 1145 (Bd. Pat. App. & Inter. 1992) ("controlled stream of fluid" provided reasonable antecedent basis for "the controlled fluid"). Inherent components of elements recited have antecedent basis in the recitation of the components themselves. For example, the limitation "the outer surface of said sphere" would not require an anteced-ent recitation that the sphere has an outer surface.

§ 2173.05(e) (emphasis added). Applicants note that claims 1, 14 and 19 each recite "a polypeptide which is immunogenic in a mammal", which one of ordinary skill would recognize as an "immunogenic polypeptide". There is therefore no need to amend claims 1, 14 and 19 in this manner, and Applicants respectfully request that the objection be withdrawn.

The Examiner has also stated that the marked-up copy of claim 18, as submitted with the Reply to the previous Office Action, is incorrect. Applicants submit herewith a correct version of claim 18. For the Examiner's convenience, the marked-up copy of claim 18 is included in the attached "Marked Up Version of the Amendments", with the amendments submitted in the previous Reply in curly brackets and double underlining (to show deletions and additions), and

the present amendments in square brackets and single underlining (to show deletions and additions).

Entry of the amendments is respectfully requested.

Claim Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-3, 5-6, 8-9, 13-14, 16 and 18-20 are rejected under 35 U.S.C. § 112, first paragraph, on the grounds that the specification "does not reasonably provide enablement for using the method to treat or prevent disease, using any protein, any mammal."

There is no requirement that an applicant for patent enable every possible embodiment for which the invention might potentially prove useful, such as use of the invention with "any protein" and in "any mammal". This very issue has been addressed by the Board of Patent Appeals of the U.S. Patent and Trademark Office in *Ex parte Mark* (12 U.S.P.Q.2d 1904 (Bd. Pat. App. & Int. 1989)). In that case, the Examiner stated that the rejected claims encompassed "any protein". The Board reversed the rejection on those grounds, noting that the specification presented working examples, and framed the relevant enablement issue as a question of whether, for a given protein containing cysteine residues, one skilled in the art (1) would be able to substitute or delete the cysteine residues as desired, and (2) could routinely determine whether deletion or replacement of cysteine residues in a given instance in fact resulted in an operative mutein falling within the description provided in the claims. The Board observed that "[t]he fact that a given protein may not be amenable for use in the present invention in that the cysteine residues are needed for the biological activity of the protein does not militate against a conclusion of enablement", and that "[o]ne skilled in the art is clearly enabled to perform such work as needed to determine whether the cysteine residues of a given protein are needed for retention of biological activity."

As in *Mark*, one of ordinary skill is fully enabled to practice the present invention commensurate in scope with the claims. The claims recite methods of regulating expression of a protein by a promoter that is in turn regulated by the concentration of a drug for which the promoter is specific. The specification sets forth examples demonstrating the use of a single

vector containing all the components of a tetracycline regulatable system (TRS) for the pharmacological regulation of a foreign gene expressed in a human T cell line. Specifically, the applicants demonstrate that a scFv-TRCζ chimeric molecule can be functionally expressed in a human T cell line and its expression can be pharmacologically down-modulated by altering the concentration of tetracycline or a tetracycline analog. One of skill in the art can readily substitute the described TRS transfected cells for given TRS transfected cells of the claimed methods, transplant the transfected TRS cells into a given mammal as is known in the art, and then regulate the expression of the transfected gene within the TRS transfected cells by modulating the level of tetracycline within the mammal, as is also known in the art.

That one of ordinary skill may use the invention to regulate a polypeptide of interest to him or her does not go to the issue of enablement of the claims. Applicants have set forth a method, demonstrated by working examples. There is no requirement that Applicants foresee every potential use to which their invention may be put and provide a working example for each.

The Office Action also states that the "claims as written encompass broad and divergent technologies", such as "methods of regulating protein expression in transgenic animals, methods of regulating protein expression in cells administered to a mammal for the purpose of therapy and methods of regulating marker protein expression in cells administered to a mammal for the purpose of monitoring the cells", etc. The Office Action implies that Applicants are required to dictate to one of ordinary skill the uses to which the invention may be put. There is no such requirement. Applicants have invented a method for regulating expression, under very specific conditions, namely, that the nucleic acid expressing the polypeptide be placed under the control of a specific kind of promoter. That one may choose to use this method to regulate protein expression in transgenic animals, or to regulate protein expression in cells, is not germane to the question of whether or not the invention is enabled.

The Office Action also states that the Applicants have not provided any arguments regarding "how to use the method or cells claimed to obtain a therapeutic effect" (page 4, line 20 and page 5, line 1 of the Office Action). Applicants respectfully disagree.

On the contrary, the present invention clearly demonstrates how the claimed methods and cells could produce a therapeutic effect. For instance, the specification states (at page 17, lines 14-19) that "[t]his invention includes methods for producing cells expressing a regulated amount of leukocyte-activating molecule on their surface. For example, the cell may be transformed with a sequence directing the drug-regulatable expression of a TCR molecule, or of a chimeric TCR molecule which comprises at least the cytoplasmic signaling domain of the TCR molecule." Example 1 (pages 22-29) teaches the generation of a human T cell line that expresses, in a tetracycline regulatable manner, a scFv-TCRζ chimeric receptor. The specification also clearly indicates that "T lymphocytes can be transformed with a nucleic acid sequence directing the tetracycline-sensitive expression of a chimeric TCR molecule having specific binding activity for a tumor associated antigen." (specification, page 18, lines 2-4). The specification further states on page 19, line 32 to page 20, line 2 that "when the disease is associated with tumor formation, the claimed invention is successful when tumor growth is arrested, or tumor mass is decreased by at least 50% and preferably 75%." The specification therefore does clearly teach how the method and cells can be used to obtain a therapeutic effect and the therapeutic embodiments of the claims consequently are enabled. The Applicants therefore request that the rejection be reconsidered and withdrawn.

Furthermore, the purpose to which one of ordinary skill chooses to put the invention is not germane to the question of enablement. As noted in MPEP § 2164.08(b), the presence of inoperative embodiments within the scope of a claim does not render a claim nonenabled. As stated by the Federal Circuit, "[i]t is not a function of the claims to specifically exclude possible inoperative substances." (*Atlas Powder Company v. E.I. Du Pont De Nemours & Company*, 224 U.S.P.Q. 409, 414 (Fed. Cir. 1984)). Absolute predictability of the activity of embodiments which may be embraced within the claims is a not a requirement of the statute. The decision in *In re Angstadt*, 190 U.S.P.Q. 218 (C.C.P.A. 1976) clearly states that every embodiment need not be disclosed, even in an unpredictable art, and clearly permits the presence of a screening step to identify those embodiments which possess the desired activity. In fact, in *Angstadt*, the Court specifically dismissed the notion that the specification must provide a level of guidance that

would predict the outcome of an experiment (or reaction) "with reasonable certainty before performing the reaction" and that "such a proposition is contrary to the basic policy of the Patent Act, which is to encourage disclosure of inventions and thereby to promote progress in the useful arts."

Whether or not one of ordinary skill chooses to use the invention to regulate protein expression in a therapeutic context, or to study regulation of marker proteins is also not relevant to the question of enablement. Applicants are not required to limit the scope of claims which are enabled because someone might possibly experience some difficulty in using the invention for a particular purpose or in a specific context. As stated above, there is no requirement for an applicant for patent to exclude inoperative embodiments, let alone those embodiments that might *possibly* prove to be inoperative.

The Office Action states that claims 1, 14 and 19 are limited to "immunogenic polypeptides" which are stated to be proteins that induce a therapeutic immune response. Applicants respectfully note that on page 12, the present Office Action characterizes LacZ and luciferase as immunogenic polypeptides (page 12, lines 2-3 and 11-12). Furthermore, claim 18 encompasses using a heterologous polypeptide. Applicants respectfully submit that a heterologous polypeptide can in principle act as a marker polypeptide. Ideally the product of a marker gene is not found naturally within the organism under study, thus allowing its expression to be traced within that organism. For this reason, marker genes may well be foreign and hence immunogenic. Applicants therefore respectfully disagree with the Examiner and submit that the specification and the art at the time of filing do indeed teach that the invention could be used to study the expression of immunogenic polypeptide genes including that of marker genes. The invention therefore does have a utility "other than gene therapy in humans".

In the instant Office Action, it is argued that the Specification fails to teach that the host had already made an immune response to the protein prior to receiving the transformed cells. Applicants respectfully disagree. Page 22, lines 2-3 of the Specification state that "the method of the invention is typically performed in a mammal which has <u>already</u> made an immune response to the immunogenic polypeptide" (emphasis added). Claims 14, 18 and 19 are amended herein

to require that the mammal have already made an immune response to the polypeptide. Claim 1 was previously amended in this way. In fact, the claims were so limited because the real advantage of the claimed invention is that one can use it to circumvent an immune response to the heterologous polypeptide. Applicants submit that initiation of an immune response to an immunogenic polypeptide and monitoring for the presence of circulating antibodies directed against said immunogenic polypeptide is well within the ability of one of ordinary skill in the art, and respectfully request that the rejection be reconsidered and withdrawn.

The Office Action also states (on page 6, lines 11-14) that "[t]he specification does not teach what effect administering the cell has on a host that has already had an immune response to the protein" and that "[t]he only disclosed purpose for administering a cell expressing a protein to a mammal after the mammal has already had an immune response to the immunogenic polypeptide is to treat cancer." Applicants respectfully disagree with both of these points.

The effect of administering cells to a host that has initiated an immune response to an expressed immunogenic polypeptide within the cells is dictated by the effect elicited by the heterologous expression of the immunogenic polypeptide gene within the transplanted cells. The specification states (at page 17, lines 1-2) that the transplanted cells are autologous or tissue matched with the recipient mammal. The Specification also teaches how heterologous gene expression can be tightly controlled by modulating the systemic level of tetracycline or an analogue within the blood stream of the host (see page 20, "Dosage and Administration"). Thus, the Specification does teach a procedure where one skilled in the art could determine the effect of administering cells into a host that has already produced an immune response to a protein that can be expressed within said cells. Applicants respectfully request reconsideration and withdrawal of the rejection.

Likewise, the Specification teaches purposes for the invention other than treating cancer. On pages 20-21 of the Specification, for instance, Applicants teach how the instant invention can be used to direct the heterologous expression of a number of therapeutic polypeptides within cells transplanted into a mammal after the mammal has already produced an immune response to the immunogenic therapeutic polypeptide. For example, on page 21, lines 3-5, the Specification

cites such polypeptides as "growth factors and blood clotting factors, hormones, neurotransmitters, enzymes, apolipoproteins, receptors drugs, oncogenes, tumor antigens, tumor suppressors, structural proteins, viral antigens, parasitic antigens and bacterial antigens." This, the disclosed purpose of the invention, is not confined solely to the treatment of cancer. Applicants therefore respectfully request withdrawal of the rejection.

The Office Action further asserts that the Specification fails to teach "the protein, level of expression, target tissue, mode of delivery or immune response required to have a therapeutic effect" (Office Action, page 6, line 16-17).

Applicants disagree. The instant invention teaches how expression of a heterologous potentially therapeutic protein can be modulated within a cell that is transplanted into a mammal that has already produced an immune response to said therapeutic protein. Applicants submit that it is well within the ability of one skilled in the art to modulate the amount of tetracycline inducer within a host and to measure a therapeutic effect for example a decrease in the size of a tumor without undue experimentation.

Furthermore, given the broad applicability of the invention with respect to "a heterologous polypeptide" and the therapeutic endpoint, it is not possible for Applicants to teach any meaningful parameters which are raised by the Examiner. Finally, and most importantly, it is not necessary to measure the level of heterologous polypeptide, gene expression, or target tissue to carry out the invention. The success of the claimed invention depends upon the success of the therapy, and this is judged on a case by case basis by a medical doctor.

The Office Action also states (at page 6, line 20 to page 7, line 2) that the specification fails to teach "transfecting an ES cell with a vector encoding an immunogenic protein and transferring the ES cell into a mammal that has had an immune response to the immunogenic protein". Applicants are making a *bona fide* attempt to respond to this particular rejection, but are genuinely confused. The claims do not specifically recite embryonic stem cells.

The Office Action then states that "[t]he methods also relate to regulating marker protein expression in a mammal; however, the specification does not teach transferring a cell to a mammal that has had an immune response to a marker protein."

However as stated above, to be useful, a marker gene may be one which is simply not expressed within the test organism and since it is foreign is immunogenic. As noted previously, lacZ, a bacterial enzyme, is a widely used marker gene that the Examiner also considers to an immunogenic polypeptide (see page 12, lines 11-12 of the present Office Action). The Applicants therefore submit that the Specification does indeed teach transferring a cell to a mammal that has had an immune response to an immunogenic polypeptide including the products of marker genes that can also be "immunogenic polypeptides". The Applicants therefore respectfully request that the rejection be reconsidered and withdrawn.

Claims 1-3, 5-6, 8-9, 13, and 18-20 are also rejected, the Office Action stating that the specification does not teach how to transfect cells *in vivo* for reasons of record.

The Office Action asserts the purpose of the method "to produce an immunogenic polypeptide *in vivo* despite the immune response" is unclear. The Applicants refer the Examiner to page 9, line 20 to page 10, line 1 of the Specification. Cell surface proteins such as tumor-associated antigens are not unique to tumor cells. They are expressed at relatively high densities on the surface of tumor cells, but may also be expressed at lower density on the surface of certain non-tumor cell types. In one embodiment, the present invention provides a means to generate T cells that are specifically tumor reactive.

The Office Action also states that the Specification does not teach the parameters required to administer cells transfected with viral vectors encoding a protein such that the protein is functionally expressed *in vivo*. Applicants again respectfully disagree. Such methods were well known as of the priority date of the instant application, and Applicants should therefore not be required to teach such well-known methods. For instance, the Examiner is referred to page 16, lines 4-11 of the specification, which cites Hofmann *et al.* (*Proc. Natl. Acad. Sci. USA* 93:5185-5190 (1996)), which describes in detail how to transform cells *in vitro* with a heterologous construct, and express the heterologous polypeptide in a mouse. There is no evidence in the art that regulation of heterologous gene expression within such viral vectors is any different from that of tetracycline regulated constructs introduced into cells using non viral

methods. Applicants, therefore, submit that the use of viral vectors in the claims is enabled as of the filing date by the specification and what was known to one of the ordinary skill in the art.

However, as requested by the Examiner, and solely to expedite prosecution, claims 1, 18 and 19 are amended to indicate that the cells are transformed *in vitro*. Applicants reserve the right and intend to pursue claims not limited to *in vitro* transformation in a divisional application. Applicants respectfully request reconsideration and withdrawal of the rejection.

Claim Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-3, 5-6, 8-9, 13-14, 16 and 18-20 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which Applicants regard as the invention.

Specifically, the Office Action states that the phrase "mammal that has made an immune response to said immunogenic polypeptide" is unclear, the reasoning being that it is unclear if the mammal has been exposed to the immunogenic polypeptide prior to the "introducing" step or if the mammal has made an immune response to the immunogenic polypeptide which is encoded by the vector.

The specification on page 4, lines 19-20 states unambiguously that "the mammal has already made an response to the immunogenic polypeptide" (emphasis added). Therefore, the cells are introduced to a mammal that has already made an immune response to the immunogenic polypeptide before the transformed cells are introduced. Applicants respectfully submit that the claims are clear on their face, and request that the rejection on this basis be reconsidered and withdrawn.

Claims 1-3, 5-6, 8-9, 13 and 18-20 are also rejected, the Office Action stating a belief that claims 1, 18 and 19 are directed to "regulating the expression of a nucleic acid sequence", but only result in expression of a nucleic acid sequence, and that the phrase is not a clear, positive step indicating that expression of the protein is altered in the presence or absence of tetracycline.

Applicants have amended claims 1, 18 and 19 to indicate the expression of the polypeptide within the transformed cells is altered in the presence or absence of tetracycline.

Claims 1-3, 5-6, 8-9, 13-14, 16 and 18-20 are rejected as indefinite, the Office Action stating that the phrase "altering the concentrations of tetracycline . . . to which the cell is exposed" is indefinite as it relates to the step of administering the cells, and that it cannot be determined when the concentration of tetracycline is altered in relationship to when protein expression is inhibited *in vitro*. The Office Action states that the claims do not clearly recite (1) when the mammal is first exposed to the immunogenic polypeptide, (2) when the immunogenic polypeptide encoded by the vector is expressed, (3) when tetracycline is first administered, (4) when the tetracycline concentration is altered, and (5) when the tetracycline concentration is altered in relationship to the first and second exposures to the immunogenic polypeptide.

The point at which the mammal is exposed to the immunogenic polypeptide has been addressed above, where it was stated that the cells are introduced to a mammal that has already made an immune response to the immunogenic polypeptide before the transformed cells are introduced.

The second point, the time of expression of the immunogenic polypeptide encoded by the vector, is not required to be known in order to carry out the invention. It is clear that expression cannot occur before transformation with the vector containing the encoded polypeptide. It is also clear that the claim requires that expression is altered in the presence or absence of tetracycline. Because expression is altered, that is, because expression can be increased or decreased, it is inappropriate to require that it be known precisely when expression occurs, as expression can be altered so as not to occur. The requirement that the time of expression be known is inconsistent with the subject matter of the claim.

Likewise, the requirement that the claims recite when tetracycline is first administered, when the tetracycline concentration is altered, and when the tetracycline concentration is altered in relationship to the first and second exposures to the immunogenic polypeptide, are also inconsistent with the subject matter of the claims. The claims recite a method of regulating the

expression of an immunogenic polypeptide. A "method of regulating" implies that one practicing the invention is granted, by practicing the invention, a measure of control (regulation) over the expression of the polypeptide. As stated in the specification, *e.g.*, at page 4, lines 10-15, the sequence encoding the polypeptide is operably linked to a promotor that is regulated by a drug, *e.g.*, tetracycline. Therefore, one practicing the invention can control the expression of the polypeptide by controlling the activity of the promoter to which it is linked, that is, by administration or withholding of the drug (*e.g.*, tetracycline).

Applicants respectfully submit that the "requirements" as outlined in the Office Action are not necessary to practice the invention, in fact, such requirements are inconsistent with the subject matter of the claims. The claims clearly state that the method of regulating the polypeptide expression is accomplished by altering the concentration of tetracycline, and one of ordinary skill would understand that the regulation is therefore a function of the alteration of the tetracycline concentration by the one of ordinary skill practicing the invention. The rejection on this basis should therefore be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 102(b)

Claims 1, 18 and 19 are rejected under 35 U.S.C. § 102(b) as anticipated by Shockett *et al.*, 1995, *Proc. Natl. Acad. Sci. USA* 92:6522-6526, and claims 14 and 16 remain rejected under 35 U.S.C. § 102(b) as anticipated by Hofmann *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:5185-5190.

Claim Rejections in View of Shockett et al.

Claims 1, 18 and 19 are rejected under 35 U.S.C. § 102(b) as anticipated by Shockett *et al.*, 1995, *Proc. Natl. Acad. Sci. USA* 92:6522-6526, the Office Action stating that the reference teaches transducing fertilized mouse eggs with a vector containing the luciferase gene under the control of a tetracycline operator, and implanting the eggs into pseudopregnant female mice. The Office Action interprets the implantation of the eggs as the equivalent of introducing the cell

into a mammal. The Office Action also notes that this reference introduces the cell into the mammal before the immunogenic response is produced.

As stated above, Applicants have amended claims 18 and 19 to recite that the mammal has already made an immune response to the polypeptide before the time at which the cell (transformed with the vector containing the nucleic acid encoding the polypeptide and the tetracycline-regulatable promoter) has been introduced into the mammal. Claim 1 already contains such a recitation.

The Office Action also notes that Claim 18 recites regulating expression of a coding sequence in a leukocyte, yet asserts that this claim is anticipated by this reference. Applicants, however, have been unable to find any teaching regarding leukocytes within this reference. Claims 18 therefore cannot be anticipated by this reference.

Applicants respectfully submit that the claims are not anticipated by Shockett *et al.*, and respectfully request that the rejection on the basis of this reference be reconsidered and withdrawn.

Claim Rejections in View of Hofmann et al.

Claims 14 and 16 are rejected under 35 U.S.C. § 102(b) as anticipated by Hofmann *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:5185-5190, the Office Action stating that the reference teaches transfection of lymphocytes with a retroviral vector encoding LacZ operatively linked to the tetracycline operator and also encoding tetR-VP16. The Office Action also states that lymphocytes are considered leukocytes.

Claim 14, as amended, recites that the mammal has already made an immunogenic response to the immunogenic polypeptide, and also recites an "autologous" leukocyte". An autologous leukocyte is one that is derived from the same organism or one of its parts. Hofmann *et al.* fails to teach introduction of such a transformed leukocyte into such a mammal, and therefore the reference fails to anticipate claim 14, and dependent claim 16. Applicants respectfully request that the rejection of these claims in view of Hofmann *et al.* be reconsidered and withdrawn.

Claim Rejections Under 35 U.S.C. § 103(a)

Claim 18 is rejected under 35 U.S.C. § 102(b) as obvious in view of Hofmann *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:5185-5190, for reasons of record, namely, that the reference teaches transfection of lymphocytes with a retroviral vector encoding LacZ operatively linked to the tetracycline operator and also encoding tetR-VP16, that lymphocytes are considered leukocytes, and that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to alter the concentration of tet thereby regulating the expression of LacZ in the lymphocytes because Hoffmann [*sic*] taught altering the concentration of tet thereby regulating expression of LacZ in myoblasts transfected with the vector encoding LacZ operably linked to a tet-regulatable promoter".

Claim 18 clearly states that the cell is introduced into a mammal, and that the concentration of tetracycline is altered after the cell is introduced into the mammal. In addition, Applicants have amended claim 18 to recite that the mammal has already made an immune response to the polypeptide before the time at which the cell (transformed with the vector containing the nucleic acid encoding the polypeptide and under the control of the tetracycline-regulatable promoter) has been introduced into the mammal. None of these aspects appear to be taught in Hofmann *et al.* The claims are therefore not anticipated by, nor can they be obvious in view of, this reference, and the rejection on this basis must be reconsidered and withdrawn.

Applicants submit that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENTS:

Specification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph at page 7, line 12 with the paragraph below, which is marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph:

> Figures 2A and 2B show [Figure 2 shows] representative results confirming the regulation of the chTCR gene expression by tetracycline analogs. In Figure 2A, stable transfected uncloned JLAV12S (left hand side) and JN3S Jurkat (right hand side) cell populations were cultured for 48 hours in tetracycline-free medium (CM, upper row of panels) or in the presence of 1 μg/ml of Tet (broken line) or Dox (solid line) (lower row of panels) and the surface expression of chTCRs was examined after staining with FITC-conjugated goat antisera to mouseλ light chain. Figure 2B shows a timecourse of inactivation of chTCR gene expression in JLAV12S cells zero hours (top left), 8 hours (top right), 12 hours (bottom left) or 24 hours (bottom right) after addition of Dox at 1 μg/ml. In both Figures 2A and 2B negative controls (FITC-conjugated goat antisera to mouse IgG) are overlaid (filled curve). The fluorescence channel number is plotted along the x axis, and the y axis represents the relative cell number.

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend claims 1, 14, 18 and 19 as follows:

1. (Four times amended) A method of regulating the expression of a recombinant nucleic acid sequence encoding a polypeptide which is immunogenic in a mammal; the method comprising introducing into a mammal that has made an immune response to said immunogenic polypeptide, a cell transformed in vitro with [comprising] a vector comprising a nucleic acid encoding said immunogenic polypeptide, operably linked to a tetracycline-regulatable promoter; and altering the concentration of tetracycline or an analog thereof to which the cell is exposed so as to achieve in said mammal expression of said immunogenic polypeptide that is altered [nucleic acid sequence as permitted] in the presence or absence of tetracycline or an analog thereof.

14. (Three times amended) An isolated autologous leukocyte transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic in [to] a mammal, and to which the mammal has already made an immune response, the nucleic acid sequence being operably linked to a tetracycline-regulatable promoter, such that expression of the immunogenic polypeptide by the leukocyte is controlled by altering the concentration of tetracycline or an analog thereof to which the leukocyte is exposed after introduction to a mammal.

Note: For the convenience of the Examiner, the marked-up copy of claim 18, below, includes the amendments submitted in the previous Reply in curly brackets and double underlining (to show deletions and additions), and the

amendments presently submitted in square brackets and single underlining (to show deletions and additions).

18. (Three Times Amended) A method of regulating the expression of a nucleic acid sequence encoding a heterologous polypeptide in a leukocyte after introduction of said leukocyte into a mammal that has made an immune response to the heterologous polypeptide, comprising transforming the isolated leukocyte in vitro with the nucleic acid coding sequence operably-linked to a tetracycline-operator sequence, and a sequence encoding a tetracycline-sensitive DNA-binding expression-regulating polypeptide; and altering the concentration of tetracycline, or an analogue thereof, to which the leukocyte is exposed after said introduction into said mammal, thereby regulating the {so as to regulate} expression of the coding sequence.

19. (Amended) A method of regulating the expression of a recombinant nucleic acid sequence encoding a polypeptide which is immunogenic in a mammal and to which the mammal has already made an immune response, the method comprising introducing[,] a cell into said mammal, said cell transformed in vitro with [comprising] a vector comprising a nucleic acid encoding said polypeptide, said nucleic acid operably linked to a tetracycline-regulatable promoter; wherein prior to introduction of the cell into the mammal the expression of the polypeptide is inhibited in vitro, and altering the concentration of tetracycline or an analog thereof to which the cell is exposed in said mammal, so as to achieve in said mammal expression of said immunogenic polypeptide that is altered [nucleic acid sequence as permitted] in the presence or absence of tetracycline or an analog thereof.